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RESEARCH ARTICLE

Comparative digesta retention patterns in ratites

Samuel Frei,¹ Sylvia Ortmann,² Christoph Reutlinger,³ Michael Kreuzer,⁴ Jean-Michel Hatt,¹ and Marcus Clauss^{1*}

¹ Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Switzerland

² Leibniz Institute for Zoo and Wildlife Research (IZW) Berlin, Germany

³ Reutlingers Straussenfarm, Rikon, Switzerland

⁴ ETH Zurich, Institute of Agricultural Sciences, Zurich, Switzerland

* Corresponding author: mclauss@vetclinics.uzh.ch

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ABSTRACT

Ratites differ distinctively in the anatomy of their digestive tracts. For example, Common Ostriches (*Struthio camelus*, hereafter Ostriches) have a particularly long, voluminous colon and long, paired caeca; Rheas (*Rhea spp.*) are characterized by a short colon with particularly prominent paired caeca; and Emus (*Dromaius novaehollandiae*) have neither prominent caeca nor a prominent colon. We tested whether digesta excretion patterns corresponded to these differences in anatomy, expecting Ostriches to have the longest and Emus the shortest digesta retention times, and Rheas possibly showing a selective retention of fluids observed in other birds and mammals with prominent caeca. We used 6 Ostriches (97–123 kg), 5 Greater Rheas (*R. americana*, 22–27 kg), and 2 Emus (32–34 kg) fed a common diet of alfalfa pellets *ad libitum* in captivity. Intake per unit of metabolic body mass did not differ between Ostriches and Greater Rheas but was significantly higher in Emus, which also displayed higher defecation frequencies and lower fiber digestibility. Mean digesta retention time for small fiber particles (2 mm) differed significantly among species (Ostrich: 30–36 h; Greater Rhea: 7–19 h; Emu: 1.3–1.8 h), but there were no differences between the retention of 2 mm or 8 mm particles or a solute marker within species. The shape of the marker excretion curves corresponded to digesta mixing in the digestive tract of Ostriches and Greater Rheas but not Emus. The calculated dry matter gut fill (% of body mass) was significantly higher in Ostriches (1.6–1.8) than Greater Rheas (0.3–1.0) and Emus (0.2). Ostriches had the highest and Emus the lowest fecal dry matter concentration. These physiological findings match the differences in digestive anatomy and support the concept that in ratites, herbivory—and hence flightlessness—evolved repeatedly in different ways.

Keywords: ratite, Ostrich, Rhea, Emu, digesta retention, digestion, intake, gut fill

Comparación de patrones de retención digestiva en ratites

RESUMEN

Las ratites se diferencian notablemente en la anatomía de sus tractos digestivos. Por ejemplo, los avestruces (*Struthio camelus*) tienen un colon particularmente largo y voluminoso y un ciego emparejado largo; los ñandúes (*Rhea spp.*) se caracterizan por un colon corto con un ciego emparejado particularmente prominente; y los emús (*Dromaius novaehollandiae*) no tienen ni un ciego muy prominente ni un colon prominente. Evaluamos si los patrones de excreción digestivos se correspondieron con estas diferencias anatómicas, esperando que el tiempo de retención digestiva en los avestruces fuera el más largo y que en los emús fuera el más corto, con los ñandúes mostrando posiblemente una retención selectiva de fluidos observada en otras aves y mamíferos con ciego prominente. Empleamos 6 avestruces (97–123 kg), 5 individuos de *Rhea americana* (22–27 kg) y 2 emús (32–34 kg) alimentados *ad libitum* en cautiverio con una dieta común de pellets de alfalfa. La ingesta por unidad de peso corporal metabólico no varió entre avestruces y ñandúes pero fue significativamente más alta en emús, quienes también mostraron frecuencias de defecación más altas y menor digestibilidad de fibras. El tiempo medio de retención digestiva para pequeñas partículas de fibra (2 mm) varió significativamente entre especies (avestruz: 30–36 h; ñandú: 7–19 h; emú: 1.3–1.8 h), pero no hubo diferencias dentro de las especies entre la retención de partículas de 2 mm u 8 mm o un marcador de soluto. Las formas de las curvas de excreción del marcador correspondieron a las mezclas de alimentos en el tracto digestivo de avestruces y ñandús pero no de emús. El cálculo del contenido de materia seca del intestino (% de peso corporal) fue significativamente más alto en los avestruces (1.6–1.8) que en los ñandús (0.3–1.0) y emús (0.2). Los avestruces tuvieron la más alta y los emús la más baja concentración de materia seca fecal. Estos hallazgos fisiológicos concuerdan con las diferencias en la anatomía digestiva y apoyan el concepto de que en las ratites, la herbivoría—y por ende la falta de vuelo—evolucionaron repetidas veces de modos diferentes.

Palabras clave: avestruz, contenido estomacal, digestión, emú, ingesta, ñandú, ratite, retención digestiva

INTRODUCTION

The term “ratites” is commonly used to designate various taxa of flightless birds of the superorder Palaeognathae. Ratite stems from the Latin “ratis” for raft, indicating a common anatomical feature in these birds: the absence of a keel (and ventral muscles) at their sternum (Fowler 1996). Although ratites share many external features, they are a heterogeneous group. Various phylogenetic relationships among ratites have been proposed, differing specifically in whether Common Ostriches (*Struthio camelus*, hereafter Ostriches) or Rheas (*Rhea* spp.) are more closely related to Emus (*Dromaius novaehollandiae*; van Tuinen et al. 1998, Cooper et al. 2001, Haddrath and Baker 2001, Mitchell et al. 2014). This phylogenetic debate also includes flighted Tinamous (*Tinamou* spp.), Cassowaries (*Casaurius* spp.), Kiwis (*Apteryx* spp.), Moas, and Elephant birds; the debate is relevant to whether flightlessness in ratites evolved only once or several times independently (Harshman et al. 2008, Phillips et al. 2010, Baker et al. 2014). Most recent results indicate a discrepancy between geographic location and phylogenetic relatedness among ratite species, suggesting that the major ratite lineages must have dispersed by flying before evolving converging anatomical characteristics and flightlessness (Mitchell et al. 2014).

Flightlessness also has implications for the digestive physiology of these lineages. A thorough microbial digestion of plant material, which necessitates a voluminous gut with long digesta retention times (Clauss et al. 2013), is usually considered incompatible with anatomic adaptations of flight. Avian herbivores that rely heavily on microbial plant fiber fermentation are therefore thought to be obligatorily flightless (e.g., Morton 1978). Even the Hoatzin (*Opisthocomus hoazin*), the only known avian foregut fermenter, is reported to be a poor flyer (Grajal et al. 1989).

Multiple origins of flightlessness in ratites could explain the distinct variation of their digestive tracts. Despite other general morphological similarities (Livezey and Zusi 2007), Ostriches, Rheas, and Emus differ distinctively in the anatomy of the digestive tract (Figure 1). Ostriches have well-developed, large, sacculated caeca and a particularly long and partly sacculated colon (Skadhauge et al. 1984, Hongo et al. 2006). In contrast, Rheas evolved particularly large, paired, sacculated caeca without a distinctive colon, and Emus show neither pronounced caeca nor a prominent colon (Cho et al. 1984, Herd and Dawson 1984).

These differences in digestive anatomy should also be reflected in differences in digestive physiology, such as time digesta is retained in the gut and relation of solute and particulate digesta excretion patterns. First, digesta retention time should increase with the length of the

digestive tract, a hypothesis supported by the large differences in retention time of ~40 h vs. 5 h so far reported between Ostriches and Emus, respectively (Herd and Dawson 1984, Swart et al. 1993, Fritz et al. 2012). Stewart (1994) claims that Rheas are similar to Ostriches in this respect but does not provide a reference. To our knowledge, corresponding data for Rheas (or Cassowaries and Kiwis) are lacking. Second, in mammals the difference in the retention times between solutes and particles varies systematically with digestive tract anatomy (Müller et al. 2011). In colon fermenters, this difference increases (with shorter solute retention times) as overall digesta retention increases. In several caecum fermenters, by contrast, solutes are retained longer than particles, which is commonly interpreted as an indication of a wash-back mechanism (Hume and Sakaguchi 1991, Franz et al. 2011). A similar pattern has been reported for one small herbivorous bird, the Rock Ptarmigan (*Lagopus mutus*; Gasaway et al. 1975), and has been suggested for another ratite with a large caeca, the North Island Brown Kiwi (*Apteryx mantelli*), based on the composition of the contents of different gut sections (Potter et al. 2006). One could therefore predict that Rheas, with their comparatively large caeca, should display longer solute than particle retention times, and that Ostriches should display a more distinct difference between solute and particle markers than Emus. The presence of such a difference between Ostrich and Emu is not supported by previous studies, however (Herd and Dawson 1984, Fritz et al. 2012); again, data for Rheas are lacking.

A morphophysiological adaptation common to herbivorous and granivorous birds is the use of a muscular gizzard filled with grit for the reduction of ingesta particle size (e.g., Moore 1999, Fritz et al. 2011). How the flow of digesta through the gizzard is regulated is still poorly understood, in particular whether a sorting mechanism exists that allows smaller particles to pass quickly while retaining larger ones (reviewed in Fritz et al. 2012). To date, the retention of different-sized particles in ratites has only been investigated in Ostriches, with ambiguous results. In some but not all animals, smaller particles were retained longer than both larger particles and solutes, which was interpreted as a potential indication of sequestration of smaller particles in the gizzard (Fritz et al. 2012).

In the present study, we tested whether differences in the digesta retention patterns in ratites corresponded to expectations based on digestive anatomy; additionally, we further investigated differences between small and large particle retention in birds by feeding a solute and different-sized particle markers to Ostriches, Rheas, and Emus maintained on the same diet.

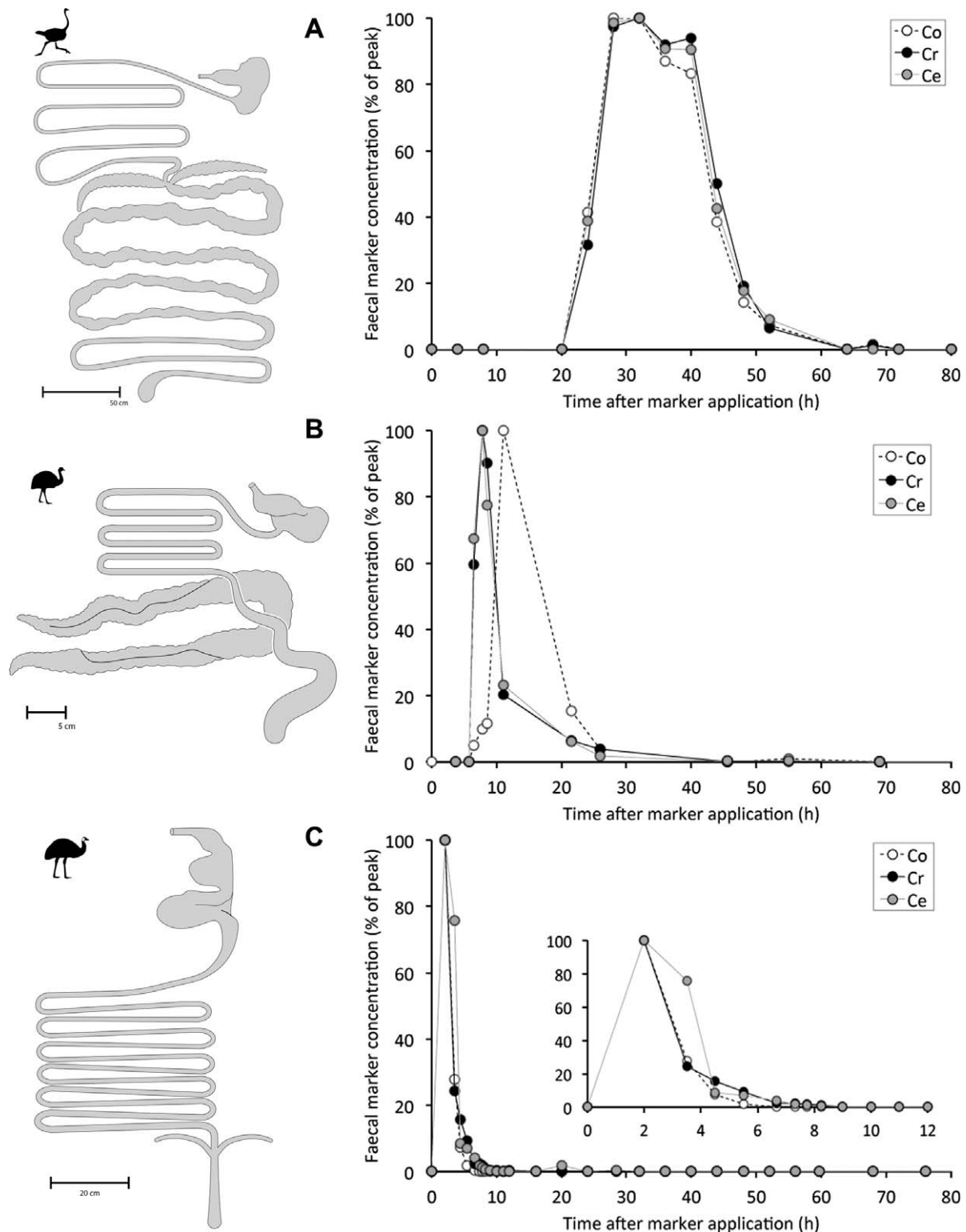


FIGURE 1. Anatomical drawings of the digestive tract of (A) Common Ostrich (*Struthio camelus*), (B) Darwin's Rhea (*Rhea pennata*), and (C) Emu (*Dromaius novaehollandiae*; redrawn from figure 3.14 in Stevens and Hume 1995), as well as exemplary marker excretion patterns for solute (Co), 2 mm particle (Cr), and 8 mm particle (Ce) markers in these species (or in the case of rheas, the closely related Greater Rhea [*Rhea Americana*]) determined in the present study (scaled to the same time interval). Inlet provided in Emus to allow closer inspection of the initial marker excretion.

TABLE 1. Nutrient composition of the alfalfa pellets* (in g kg⁻¹ dry matter).

Species	Ostrich	Greater Rhea/Emu
Organic matter	878	883
Crude protein	162	177
Ether extract	16	21
Neutral detergent fiber	424	418
Acid detergent fiber	320	330
Acid detergent lignin	83	77

*No. 2805, Provimi Kliba SA, Kaiseraugst, Switzerland (pellets produced as one batch, bagged; differences reflect variation in composition between individual bags).

METHODS

The study was performed in July and August 2013 in central Switzerland at a commercial Ostrich farm and at a private Rhea/Emu breeder, both in the vicinity of Zurich. Captive-bred animals were kept at low densities on spacious outdoor pastures (6 Ostriches per 2000 m², 8 Greater Rheas [*Rhea Americana*] per 1000 m², and 2 Emus per 4000 m²) with constant access to indoor shelter (6 Ostriches per 160 m², 8 Greater Rheas per 20 m², and 2 Emus per 20 m²) and *ad libitum* access to water and supplemental food. Ambient temperatures ranged between a maximum of 32°C during the day and a minimum of 8°C at night. Six Ostriches, 6 Greater Rheas, and 2 Emus were available for this study from private breeders. All animals were adult females. All animals were kept for an adaptation period of 14 days (on enclosures of soil or woodchips) and the subsequent 7-day experiment on an exclusive diet of pelleted alfalfa. The analyzed nutrient composition of the batches used is listed in Table 1. Alfalfa and water were provided *ad libitum*, with no access to other food items in their enclosures. Animals were weighed at the end of the experiment.

Animals were kept individually in well-ventilated enclosures for the last 3 days of the adaptation period and the 7-day collection period. Although kept individually, they had visual, acoustic, and (through the enclosure fencing) physical contact with conspecifics. Experimental enclosures were 16 m² for Ostriches and 12 m² for Greater Rheas and Emus. The birds were protected against direct sunlight, rain, and wind, and the floors were covered with carpet to facilitate fecal collection. Enclosures were under constant visual surveillance. All animals were habituated to human presence and did not seem overtly nervous to the regular removal of feces; in particular, it was possible to check for feces without arousing the animals (and thus triggering defecation; Herd and Dawson 1984).

Food intake was determined by weighing the offered amount of pelleted food and collecting the leftovers at the next feeding. This took place, at the latest, after 24 h, but sometimes earlier because *ad libitum* feeding conditions

were guaranteed by always replacing food before the feeding bowl was empty. Leftovers were dried at 60°C to constant mass to correct for potential increases in moisture content while on offer. Representative samples of the pellets were taken regularly for a pool sample submitted to standard nutrient analyses (AOAC 1995) for dry matter and total ash (AOAC no. 942.05), crude protein (AOAC no. 977.02), and ether extracts (AOAC no. 963.15) as well as neutral detergent fiber (NDF), acid detergent fiber, and acid detergent lignin analysis (Van Soest et al. 1991). All fiber values are expressed without residual ash. Analyses were performed in duplicate.

We used markers similar to a previous study on Ostriches (Fritz et al. 2012), with cobalt (Co)-EDTA as solute marker for the fluid digesta component and hay particles of different sizes (either 2 or 8 mm) mordanted with either chromium (Cr) or cerium (Ce) as particle markers, respectively. Co-EDTA and Cr-mordanted fibers were prepared according to Udén et al. (1980) and Ce-mordanted fibers according to Schwarm et al. (2008, 2009). The mordanted fibers contained 36.9 g Cr kg⁻¹ dry matter (DM) and 17.3 g Ce kg⁻¹ DM, respectively. Markers were offered with a defined portion of the pelleted feeds. The pellets soaked up the Co-EDTA solution. Particle markers were made to stick to the pellets by using carefully dosed small amounts of water. The animals had been habituated to moistened pellets during the adaptation period. Ostriches, Rheas, and Emus received 1, 0.5, and 0.5 g of Co-EDTA, and 7, 5, and 3 g each of Cr- and Ce-mordanted fiber as a pulse dose, respectively. Food with markers was offered for 60 min, except for Emus, where it was offered for 30 min, removed, and replaced with non-labelled pellets. Not all animals consumed the total amount of marked pellets, resulting in the exclusion of one Greater Rhea, and the intake of the small particle marker was too low in one Ostrich to yield a reliable signal.

Prior to marker feeding, fecal samples were taken for assessing the background levels of Co, Cr, and Ce. After marker feeding, feces were sampled at 4 h intervals during the first 2 days, at 6 h intervals on day 3, and at 8 h intervals on days 4 to 7. In addition, to prevent animals from stepping in their own feces, feces were generally removed completely when they were spotted during control. The total mass of all removed feces was noted. Individual fecal samples were taken for marker analysis, and a representative pooled fecal sample (10% of each individual defecation) was collected for nutrient analysis. For the first 24 h, all available individual defecations were analyzed separately for marker composition. For the subsequent days, samples were pooled according to the sample plan mentioned above. During sampling, droppings were carefully separated from pasty uric acid excretions. All samples were immediately dried at 60°C to constant

mass and sealed in watertight plastic bags before being ground to 0.75 mm with a centrifuge mill (Retsch GmbH, Haan, Germany).

Marker analysis was performed based on previous studies (Lechner et al. 2010). Briefly, for wet ashing we heated samples with 4 mL nitric acid and 2 mL hydrogen peroxide with the microwave MLS 'START 1500' (MLS GmbH, Leutkirch, Germany). Temperature was increased over 15 min to 170°C and over 20 min to 200°C, then held at 200°C for 5 min. The wave length was 12.25 cm and the frequency 2.45 GHz. Determination of Co, Cr, and Ce in the sample digests was performed using an inductively coupled plasma optical emission spectrometer (model Optima 8000, Perkin Elmer, Rodgau, Germany). Sample introduction was carried out by using a peristaltic pump connected to a Meinhard nebulizer with a cyclon spray chamber. The measured spectral element lines were Co: 228.616 nm; Cr: 267.716 nm; and Ce: 413.764 nm. The radio frequency power was set to 1400 W, the plasma gas was 8 L argon min⁻¹, and the nebulizer gas was 0.6 L argon min⁻¹.

The mean retention time (MRT) through the whole digestive tract was calculated according to Thielemans et al. (1978) as:

$$\text{MRT} = \frac{\sum t_i C_i dt_i}{\sum C_i dt_i},$$

with C_i = marker concentration in the fecal samples from the interval represented by time t_i (h after marker administration, using the midpoint of the sampling interval), and dt_i = the interval (h) of the respective sample

$$dt_i = \frac{(t_{i+1} - t_i) + (t_i - t_{i-1})}{2}.$$

The marker was assumed to have been excreted completely once the fecal marker concentrations were similar to the background levels determined in pre-dose fecal samples. The selectivity factor (SF) was calculated by all possible MRT ratios of individual markers.

Pooled fecal samples were analyzed for DM and NDF content. The apparent digestibility (aD) for DM and NDF was calculated as the percentage of the respective intake not excreted via feces (Robbins 1993).

The indigestible DM gut content (indDMC, kg) and the total DM gut content (DMC, kg) were calculated according to Holleman and White (1989):

$$\text{indDMC} = F \times \text{MRT},$$

with F (feces output, kg h⁻¹ DM) = total daily feces output 24 h⁻¹ and with MRT (h; MRT_{Cr} was used here). Total DMC was calculated as the sum of digestible DMC and indDMC, assuming a linear absorption of ingested food

with time spent in the digestive tract (note that this does not mean linear absorption along the digestive tract):

$$\text{DMC}_{\text{lin}} = \text{indDMC} + \left[\left(\text{indDMC} \times (\text{aD DM} \times 100^{-1}) \right) \times \left(2 \left(1 - (\text{aD DM} \times 100)^{-1} \right) \right)^{-1} \right].$$

Intraspecific comparisons (such as between different markers) within Ostriches or Greater Rheas were evaluated using paired *t*-tests. Comparisons between species were made with one-way ANOVA and Sidak post hoc tests. Statistical analyses were performed in SPSS 21.0 (SPSS Inc., Chicago IL, USA), with the significance level set to $P < 0.05$. For comparison of our results with previously reported data for mammals and birds, the data summarized in Fritz et al. (2012) were used.

RESULTS

Although the pelleted food had been produced in one batch, differences among the individual bags used led to slight differences in the nutrient composition of the diet fed to Ostriches compared to Greater Rheas and Emus (Table 1). As expected, the Ostriches were significantly heavier than the Greater Rheas and Emus (Table 2). Between Greater Rheas and Emus, body mass did not differ significantly, probably a consequence of the low sample size of Emus in the present study. In contrast to this body size difference, absolute (in g d⁻¹) and relative (in g per unit metabolic body mass and day) food intake was highest in Emus, whereas relative intake was similar between Ostriches and Greater Rheas. Similarly, Emus had particularly high relative DM excretion rates. Emus had a particularly high defecation frequency, whereas Greater Rheas often defecated only 3 times per day. In those Greater Rheas where higher defecation frequencies were noted, they seemed, subjectively, to excrete several smaller portions in rapid succession in a similar amount that their conspecifics excreted as one portion. Ostriches and Greater Rheas had distinct night rest periods during which they did not feed and did not produce feces. In contrast, Emus also produced feces during nighttime, even though they remained in a resting position and did not feed. Fecal DM concentrations were highest in Ostriches and lowest in Emus.

Ostriches had the longest and Emus the shortest MRT (Figure 1; Table 2). Emus showed a marker excretion with a rapid increase and decrease of fecal marker concentration, suggesting the absence of digesta mixing in the gastrointestinal tract (Figure 1). In contrast, the excretion patterns in Ostriches indicated a certain degree of digesta mixing due to a more gradual increase and decrease and a more extended excretion peak; the pattern for Greater

TABLE 2. Average (mean \pm standard deviation) differences among ratite species in various traits describing body mass, intake, digestion, and excretion.

	Common Ostrich ($n = 6$) <i>S. camelus</i>	Greater Rhea ($n = 5$) <i>R. americana</i>	Emu ($n = 2$) <i>D. novaehollandiae</i>
BM (kg)	109 \pm 9 ^a	24 \pm 2 ^b	32;34 ^b
DMI			
g d ⁻¹	1604 \pm 485 ^a	628 \pm 291 ^b	2591;3664 ^c
g BM kg ^{-0.75} d ⁻¹	48 \pm 16 ^a	59 \pm 28 ^a	193;263 ^b
DME			
g d ⁻¹	1197 \pm 202 ^a	293 \pm 170 ^b	1431;2156 ^c
g kg ^{-0.75} d ⁻¹	36 \pm 6 ^a	28 \pm 16 ^a	106;155 ^b
Defecations (n d ⁻¹)	13.3 \pm 2.0 ^a	4.5 \pm 2.3 ^b	15;19 ^a
Fecal DM (%)	32.2 \pm 3.1 ^a	20.1 \pm 1.3 ^b	14.3;14.4 ^c
MRT (h)			
Solutes (Co)	29.6 \pm 5.7 ^a	13.7 \pm 7.3 ^b	1.3;1.5 ^b
2 mm particles (Cr)	32.7 \pm 2.8 ^a ($n = 5$)	11.8 \pm 4.5 ^b	1.3;1.8 ^c
8 mm particles (Ce)	30.6 \pm 4.7 ^a	12.2 \pm 4.4 ^b	1.2;1.3 ^c
SF			
Cr to Co	1.04 \pm 0.10 ($n = 5$)	0.90 \pm 0.11	0.87;1.38
Ce to Co	1.04 \pm 0.12	0.97 \pm 0.29	0.80;1.00
Ce to Cr	0.99 \pm 0.07 ($n = 5$)	1.07 \pm 0.24	0.72;0.92
aD (%)			
DM	44 \pm 8 ^a ($n = 3$)	59 \pm 2 ^b ($n = 4$)	43;47 ^a
NDF	48 \pm 2 ^a ($n = 3$)	46 \pm 4 ^a ($n = 4$)	22;28 ^b
Gut fill (DM)*			
kg	1.74 \pm 0.09 ^a ($n = 3$)	0.17 \pm 0.06 ^b ($n = 4$)	0.15;0.16 ^b
% of BM	1.69 \pm 0.10 ^a ($n = 3$)	0.73 \pm 0.28 ^b ($n = 4$)	0.47;0.47 ^b

aD = apparent digestibility; BM = body mass; DM = dry matter; DME = dry matter excretion; DMI = DM intake; MRT = mean retention time; NDF = neutral detergent fiber; SF = selectivity factors of different marker combinations.

Within lines, superscripts (a, b, c) indicate significant differences ($P < 0.05$) among species (one-way ANOVA with Sidak post hoc test); there were no differences in MRT between markers within species.

* Estimated according to Holleman and White (1989) based on food intake, digesta retention and digestibility; by definition, this excludes grit.

Rheas was intermediate. In 2 Ostriches, the small particle marker excretion peak indicated a certain delay of this marker only (Figure 2A). MRT_{solute} was numerically but not significantly shorter than MRT_{particle} in Ostrich ($t = -0.809$, $P = 0.464$) and numerically but not significantly longer than MRT_{particle} in Greater Rhea ($t = 1.452$, $P = 0.220$; Table 2), even though some marker excretion curves in Greater Rheas suggested that the fluid parts of digesta might move separately from the particles in this species (Figure 1). Between the small and large particles, no difference in MRT was evident (Figure 1; Table 2). Correspondingly, all selectivity factors calculated were close to 1.0 and did not differ significantly within or between species.

Digestibility estimates were only possible in 3 Ostriches, 4 Greater Rheas, and the 2 Emus. The composition of the feces of the other animals indicated that nonfood items, including soil, stones, or woodchips, were excreted and affected the results. Coprophagy was observed repeatedly in only one Ostrich; the excretion curve of this animal showed a secondary peak for all passage markers (Figure 2B). Those digestibility measurements that could be

eventually used indicated a similar DM digestibility in Ostriches and Emus but a higher one in Greater Rheas, whereas fiber digestibility was similar in Ostriches and Greater Rheas and lower in Emus (Table 2). Ostriches had the highest relative DM gut fill and, although Greater Rheas were numerically intermediate in this respect they did not differ significantly from Emus.

DISCUSSION

The results of this study allow characterizing Rheas in comparison to Ostriches and Emus and suggest an intermediate position for Rheas in terms of digesta retention time and gut fill. Given the small sample size of this study (with only 5 Greater Rheas and 2 Emus), the results need to be considered with caution. Note, however, that the results on retention times in those 2 species for which previously published data exist, the Ostrich and the Emu (Herd and Dawson 1984, Swart et al. 1993, Fritz et al. 2012), correspond to the findings of our study, and that any limitations affected all species equally, making interspecific comparisons possible. In particular, rather

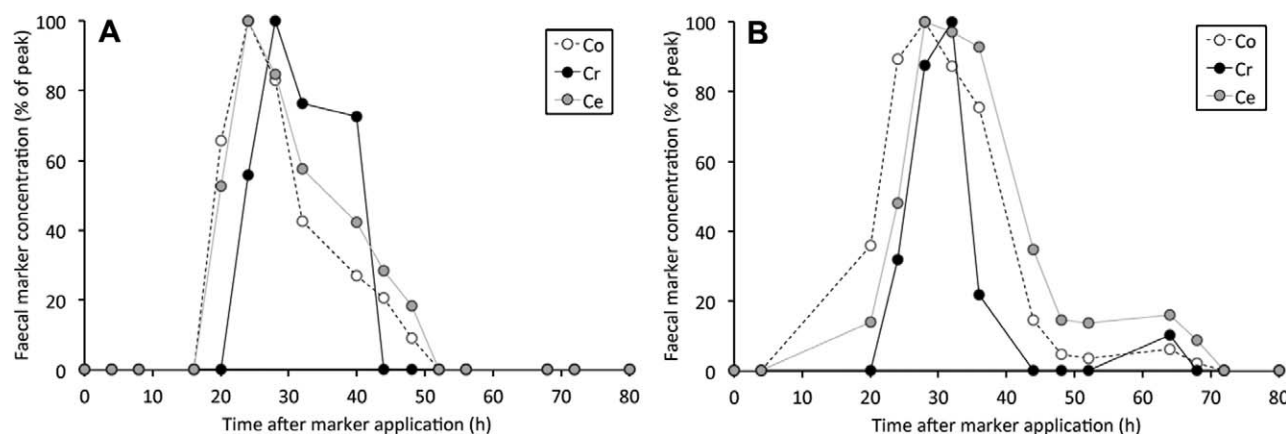


FIGURE 2. Marker excretion patterns for solute (Co), 2 mm particle (Cr), and 8 mm particle (Ce) markers in 2 individual Ostriches. (A) individual displaying a delay in small particle marker peak; (B) individual displaying a secondary marker peak indicative of coprophagy.

than relying on fecal collection from enclosure floors (even though covered by carpets), the use of a harness system as described by Bennett et al. (2012) would have been preferable. Note that effects of differences in particle size reduction, which may occur on natural foods due to the scaling with body mass (Fritz et al. 2011), did not affect the results of the present study because a common diet was used that had been finely ground before pelleting.

The marker excretion curves obtained in the present study can be linked to concepts of guts as chemical reactors (Penry and Jumars 1987, Caton and Hume 2000). The simple patterns of the Emus correspond to a plug-flow reactor with no mixing of the contents (Jumars 2000), as is also evident from the simple, tubular gut structure of this species (Figure 1). In contrast, the excretion patterns in Rheas and Ostriches, with both a more gradual increase and decrease of the excretion curve, correspond to a series of stirred tank reactors with a higher degree of digesta mixing (Jumars 2000), as to be expected from the more voluminous gut structures of these species (Figure 1). Given that voluminous guts that allow long retention times are usually considered a precondition for the successful exploitation of plant fiber as a nutritional strategy (Stevens and Hume 1998), the known difference between Ostriches and Emus matches the difference in the natural diet reported for these species. Whereas Ostriches are considered strict herbivores (Williams et al. 1993, Cooper and Palmer 1994, Milton et al. 1994), the natural diet of Emus reportedly also contains fruits, seeds, and insects in proportions that exceed those expected from accidental ingestion (Long 1965, Davies 1978, Dawson et al. 1984, Calviño-Cancela et al. 2006, Mills et al. 2008, Dunstan et al. 2013). Nevertheless, these reports also indicate that at times, Emus ingest plant material only. The comparison between Ostriches and Emus thus resembles that of mammalian herbivores with a distinct fermentation

chamber and long retention times in comparison to giant pandas (*Ailuropoda melanoleuca*) with their unspecialized guts, pronounced high food intake, particularly short digesta retention, and low fiber digestibility (Dierenfeld et al. 1982). In addition, Emus may have a low metabolism to compensate for the lack of extensive plant fiber fermentation (Calder and Dawson 1978, Dawson and Herd 1983), but comparative data for ratites do not suggest a difference between Ostriches, Rheas, and Emus in this respect (Crawford and Lasiewski 1968, McNab 2009).

The particularly short retention times of the 2 Emus in this study match their exceptionally high food intakes, which are higher than previously reported for this species (Herd and Dawson 1984, O'Malley 1996, Blache and Martin 1999; Figure 3A). This high food intake is most likely explained by the lower fiber content of the diets used previously in Emu experiments and because the Emus were assessed at a time of year when food intake was expected to be at its seasonal maximum (Blache and Martin 1999). This high food intake occurred despite observing that the Emus, like the Ostriches and Greater Rheas in the present study, did not feed at night. Uninterrupted nighttime resting periods spent in the typical ratite resting position (Immelmann 1959, 1960, Raikow 1968) have been previously described for free-ranging Ostriches (Williams et al. 1993) and captive Ostriches and Emus (Dawson et al. 1984, Degen et al. 1989). Similarly, Blache and Martin (1999) reported no feeding activity in their Emus during darkness, and Herd and Dawson (1984) indicated that Emus were not active at night and usually did not defecate during the night period. Our Emus (but not Ostriches and Greater Rheas) were observed to defecate in their typical resting position (and did not feed or even move when these feces were removed at night). In contrast, Immelmann (1960) observed Emus at a zoo to sporadically rise, feed, and defecate at night. In

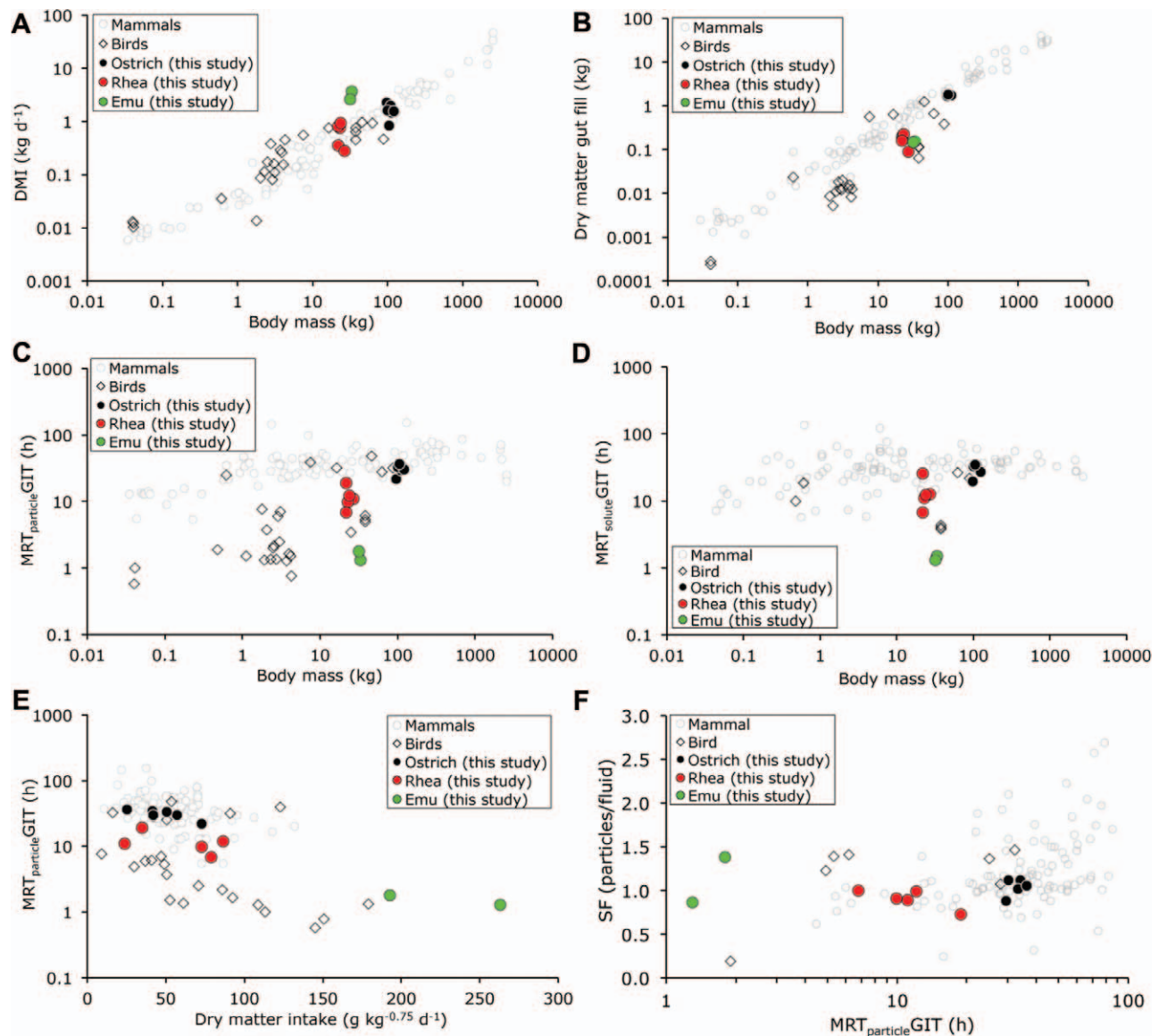


FIGURE 3. Comparative depiction of the results found in the present study for Ostriches ($n = 6$), Rheas ($n = 5$), and Emus ($n = 2$) as well as data from mammals and birds (from sources given in Fritz et al. 2012). Relationship of body mass with (A) dry matter intake (DMI), (B) dry matter gut fill, (C) particle (2 mm) mean retention time (MRT), and (D) solute (fluid) MRT; (E) relationship of relative dry matter intake with particle MRT; (F) relationship of particle MRT with the selectivity factor (SF) of small particles vs. fluid. Note the similarity of the Ostrich to previously published data, the deviating position of Emus, the intermediate position of Rheas (especially in E), and the absence of a change in SF with MRT in the entire ratite sample in F.

contrast to many herbivorous mammals, these herbivorous birds, as well as poultry and waterfowl (Mench 2009), are basically inactive at night yet have similar overall daily food intakes (Figure 3A), a phenomenon that has, to our knowledge, rarely been addressed yet could have implications for fundamental differences between mammals and birds, such as in the physiology of sleep (Roth et al. 2006, Lesku et al. 2011). Assuming that most activity budgets of free-ranging animals are limited to daytime observations, this difference might also underlie the finding by Van Gils et al. (2007) that foraging times in birds are longer than in

mammals. Whether the overall foraging times of herbivorous birds and mammals differ when 24 h observations are made remains to be investigated.

Fritz et al. (2012) described several kinds of secondary marker peaks in the Ostriches they examined. One kind affected all markers, with the second peak lower than the first, and could hence be explained by coprophagy, which was also observed sporadically in their animals. Another kind only affected the small particle marker, and Fritz et al. (2012) speculated that this might have been an effect of sequestration of small particles in the gizzard. Because this

was not accompanied by a secondary peak of the solute marker, sequestration in the caeca was considered unlikely. Additionally, they observed secondary peaks of all markers that were higher than the primary peak and could therefore not be explained by coprophagy. This suggested a discontinuous excretion of marker from the gizzard. In the Ostriches of the present study, only one secondary marker peak was observed in the animal observed to ingest its own feces (Figure 2B); potentially, the more homogeneous mix of markers and the pelleted food in the present study (as compared to the chopped alfalfa used by Fritz et al. 2012) prevented a more distinct separation of diet and markers in the gizzards. In 2 other animals, the peak of the smaller particle marker occurred slightly later than that of the other markers (Figure 2A), suggesting that some segregation of smaller diet particles in the gizzard might actually occur but does not necessarily have to be considered a regular feature. Given the overlap of levels of intake, digesta retention, and gut fill of Ostriches with that of many herbivorous mammals (Figure 3), the Ostrich can be considered similar in terms of its digestive strategy to mammalian hindgut fermenters.

Rheas are reported to be herbivores, with only low insect ingestion by adults (Martella et al. 1996, Pereira et al. 2003, Comparatore and Yagueddu 2007, Paoletti and Puig 2007, Puig et al. 2013) but more in juveniles (Schetini de Azevedo et al. 1996). Compared to Ostriches and Emus, Rheas appear to be intermediate in the present study, both in terms of digesta retention times and the derived gut fill (Figure 3). With these characteristics, they also hold an intermediate position between flying avian herbivores and Ostriches, whereas Emus resemble flying birds. With their smaller guts, Rheas achieve a fiber digestibility similar to Ostriches, despite the shorter digesta retention, which indicates that their digestive strategy might be somewhat more efficient. Details of this difference, however, remain to be explored. One of the major limitations in using Rheas for digesta passage studies is their low defecation frequency, which may make small differences in the excretion of different markers more difficult to detect. Whether the hypothesis about the longer fluid retention in Rheas has to be rejected for this reason, or because there actually is no such mechanism, can therefore not be determined.

A retrograde uric acid transport (“urinary reflux”) from the cloaca to the caeca has been suggested for various bird species, including Emus (Skadhauge 1981, Braun and Campbell 1989, Laverty and Skadhauge 2008). The anatomy of Rheas and Emus, with their comparatively short colons, seems to facilitate such a mechanism, whereas its presence seems unlikely in Ostriches due to their long colon (Skadhauge et al. 1984) and to date has not been demonstrated using contrast medium studies in Ostriches (Duke et al. 1995). If this retrograde transport

was achieved by a fluid flow, the solute passage marker could be expected to follow along, with a corresponding pattern of a selective retention. The absence of such evidence, even in Emus with their particularly high defecation frequency, would suggest that this retrograde transport occurs with only minimal involvement of fluid, or that it hardly affects the direction of the net fluid movement. In Ostriches, the long colon reduces fecal water losses, as is evident when comparing fecal DM among the 3 ratite species (Table 2).

The high DM content of Ostrich feces and the low DM content of Emu feces found in the present study correspond to values observed in free-ranging animals (Skadhauge et al. 1984, Skadhauge and Maloney 1991). In a similar manner, the length of the colon was reported to correlate with fecal DM content in ruminants (Woodall and Skinner 1993). With respect to the movement of the solute marker, the absence of an increase in the selectivity factor ($MRT_{\text{particle}}:MRT_{\text{solute}}$ ratio) with increasing digesta retention in birds (Figure 3F) contrasts with most mammalian herbivores but resembles the situation in primates (Müller et al. 2011), possibly due to a comparatively low saliva production, as presumed for birds (Klasing 1999). Comparative studies quantifying avian saliva production, however, are lacking to our knowledge.

The few studies investigating the effect of particle size and particle density on the passage through the gizzard were reviewed by Fritz et al. (2012). As reported by these authors, the results of the present study do not indicate a differential retention of large and small fiber particles in the gizzard of Ostriches (Figure 1). Evidently, the gizzard in Emus retains large, dense objects such as gastroliths or other larger objects such as wheat grains, plastic buttons (“pseudoseeds”), or glass marbles longer than the MRTs measured for the particle markers of the present study (Davies 1978, Willson 1989). With respect to fibrous plant particles, however, gizzard processing potentially does not rely on the same or a similar sorting mechanism, but passes on the digesta in the sequence that it arrives (this study) or as affected by other processes unrelated to a deliberate digesta sorting (Fritz et al. 2012).

Features of physiology and soft tissue anatomy mostly play a minor role in reconstructing phylogenies but have sometimes been used in conjunction with molecular and skeletal data (e.g., O’Leary et al. 2013, Clauss 2014). For mammals, detailed morphological soft tissue analyses, such as lung anatomy (Wallau et al. 2000) or anatomy of the gastrointestinal tract (Langer 2001), can be used to derive phylogenies, but we are not aware of cases where such characteristics have been used to actually resolve a phylogenetic debate. For birds, characteristics of the gastrointestinal tract have been included in a large-scale morphology-based phylogeny reconstruction (Livezey and Zusi 2007) but represented only ~0.5% (16 of 2945) of the

characters used (Livezey and Zusi 2006). When compared to the 2 speciation models presented by Mitchell et al. (2014), which are based on continental vicariance (i.e. assuming nonflighted dispersal that follows the sequence of the separation of landmasses from Gondwana) and a phylogeny based on mitochondrial sequence data, the digestive anatomy and physiology of ratites match the phylogeny-based model more parsimoniously than the continental vicariance model. In both models, Ostriches separated first from the common ratite lineage, which corresponds to their most-derived gastrointestinal anatomy and physiology also documented in the present study. In the model of continental vicariance, Rheas are nested within the group consisting of Kiwis, Cassowaries, and Emus as a sister group to Tinamous (figure 3B in Mitchell et al. 2014). This would mean that their “intermediate” gastrointestinal anatomy and physiology evolved recently from among ancestors that otherwise gave rise to birds of comparatively simple gastrointestinal tracts (Emus and Cassowaries: Cho et al. 1984; Tinamous: Chikilian and De Speroni 1996; Kiwis: Potter et al. 2006) and digestive physiology (this study for Emus), and as a sister group to the only extant flighted ratites. In contrast, the phylogeny based on mitochondrial sequences suggests that after the divergence of Ostriches from the ancestral ratite lineage, Rheas diverged next, with a common ancestor for all remaining ratite taxa (figure 3C in Mitchell et al. 2014). This scenario matches the intermediate position of Rheas in terms of gastrointestinal anatomy, digesta retention, and gut volume. Additionally, this scenario combines all ratites with a comparatively simpler gastrointestinal anatomy in one group with a common ancestor, which is in better line with the finding of Lavin et al. (2008) that closely related avian taxa have a similar intestinal morphology.

To conclude, the digesta excretion patterns observed in the present study correspond to the digestive anatomy of the 3 investigated ratite species, support the concept that different pathways in the evolution of ratite herbivory (and hence flightlessness) occurred, and match a recent ratite phylogeny (Mitchell et al. 2014). The results can also be helpful for assessing the impact of ratites as seed dispersers (Miller 1996, Calviño-Cancela et al. 2006, Renison et al. 2010, Schetini de Azevedo et al. 2013).

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